Table 1: Summary of DoE factors and responses in the fractionation of SBP. The total monomeric and oligomeric sugars in the soluble fraction are shown as the percentage of each sugar released from the total available in the starting material. Experiments are shown in standard order.

	Factors				Responses					
Exp	**Time (A)**	**Pressure (B)**	**Insoluble fraction recovered**	**Soluble fraction recovered**	**% soluble arabinose released**		**% soluble galacturonic acid released**		**% soluble glucose released**	
	(min)	**(Bar)**	**(g)**	**(ml)**	**Monomer**	**Oligomer**	**Monomer**	**Oligomer**	**Monomer**	**Oligomer**
1	1.00	4.00	9.60	275	0.1%	9.7%	0.0%	38.9%	0.2%	2.1%
2	30.00	4.00	5.04	670	4.8%	74.9%	0.0%	46.9%	1.5%	2.3%
3	1.00	8.00	8.61	435	0.6%	33.0%	0.0%	47.6%	0.4%	3.2%
4	30.00	8.00	4.00	690	36.8%	59.7%	8.1%	10.7%	2.3%	7.8%
5	-5.00*	6.00	50.00	-	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
6	36.00	6.00	4.76	585	19.1%	61.9%	6.4%	10.3%	2.1%	4.3%
7	15.50	3.00	9.13	295	0.5%	27.6%	0.0%	46.3%	0.5%	2.2%
8	15.50	9.00	4.45	570	12.3%	69.6%	1.8%	23.8%	2.0%	2.7%
9	15.50	6.00	5.04	470	5.2%	59.6%	0.0%	29.7%	1.4%	1.7%
10	15.50	6.00	4.93	560	5.1%	73.8%	0.0%	44.6%	1.7%	2.1%
11	15.50	6.00	5.15	410	7.5%	76.4%	0.0%	35.1%	2.0%	4.6%

*This value was adjusted to zero minutes for the purpose of the experiment.

Table 2: Factors influencing arabinose release during pre-treatment. ANOVA table for Response Surface Reduced Quadratic model based on the response data reported in Table 1.

Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	**p-value Prob > F**
Model	10921.75	4	2730.44	31.33	0.0001
A-Time	*9416.14*	*1*	*9416.14*	*108.05*	*< 0.0001*
B-Pressure	*1613.48*	*1*	*1613.48*	*18.52*	*0.0036*
A^2	*1386.47*	*1*	*1386.47*	*15.91*	*0.0053*
B^2	*331.45*	*1*	*331.45*	*3.80*	*0.0921*
Residual	610.00	7	87.14		
Lack of Fit	*412.82*	*5*	*82.56*	*0.84*	*0.6232*
Pure Error	*197.18*	*2*	*98.59*		
Cor Total	11531.75	11			

Table 3: Factors influencing galacturonic acid release during pre-treatment. ANOVA table for Response Surface Reduced Quadratic model based on the response data reported in Table 1.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**
Model	1341.29	2	670.65	4.57	0.0426
AB	*1023.36*	*1*	*1023.36*	*6.98*	*0.0268*
A^2	*685.51*	*1*	*685.51*	*4.68*	*0.0589*
Residual	1319.61	9	146.62		
Lack of Fit	*1201.58*	*7*	*171.65*	*2.91*	*0.2796*
Pure Error	*118.03*	*2*	*59.01*		
Cor Total	2660.91	11			

Table 4: Factors influencing glucose release during pre-treatment. ANOVA table for Response Surface Reduced Quadratic model based on the response data reported in Table 1.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**
Model	56.07	3	18.69	10.01	0.0044
A-Time	*39.93*	*1*	*39.93*	*21.38*	*0.0017*
B-Pressure	*14.78*	*1*	*14.78*	*7.91*	*0.0227*
AB	*9.42*	*1*	*9.42*	*5.05*	*0.0549*
Residual	14.94	8	1.87		
Lack of Fit	*7.82*	*6*	*1.30*	*0.37*	*0.8565*
Pure Error	*7.12*	*2*	*3.56*		
Cor Total	71.00	11			

Table 5: Summary of statistical analysis performed on the models for arabinose, galacturonic and glucose release.

	Arabinose	**Galacturonic Acid**	**Glucose**
Std. Dev.	9.34	12.11	1.37
Mean	59.06	34.53	4.21
C.V. %	15.81	35.07	32.47
PRESS	2038.18	2702.79	35.24
R-Squared	0.9471	0.5041	0.7896
Adj R-Squared	0.9169	0.3939	0.7107
Pred R-Squared	0.8233	-0.0157	0.5037
Adeq Precision	16.265	4.207	9.689

Fig.1 Legend

Surface response diagram showing percentage of arabinose solubilised as a function of pressure (Bar) and time (min). The optimum conditions and % solubilisation of arabinose and glucose as predicted by the model (5.3Bar for 24.4min) are highlighted in bold. The red data points represent actual experimental design points. Response surface plotted according to Equation 1. Optimisation criteria were to maximise the yield of arabinose (>70%) and minimise the yield of glucose (<6%) in the soluble fraction, thereby retaining the cellulose in the insoluble fraction.

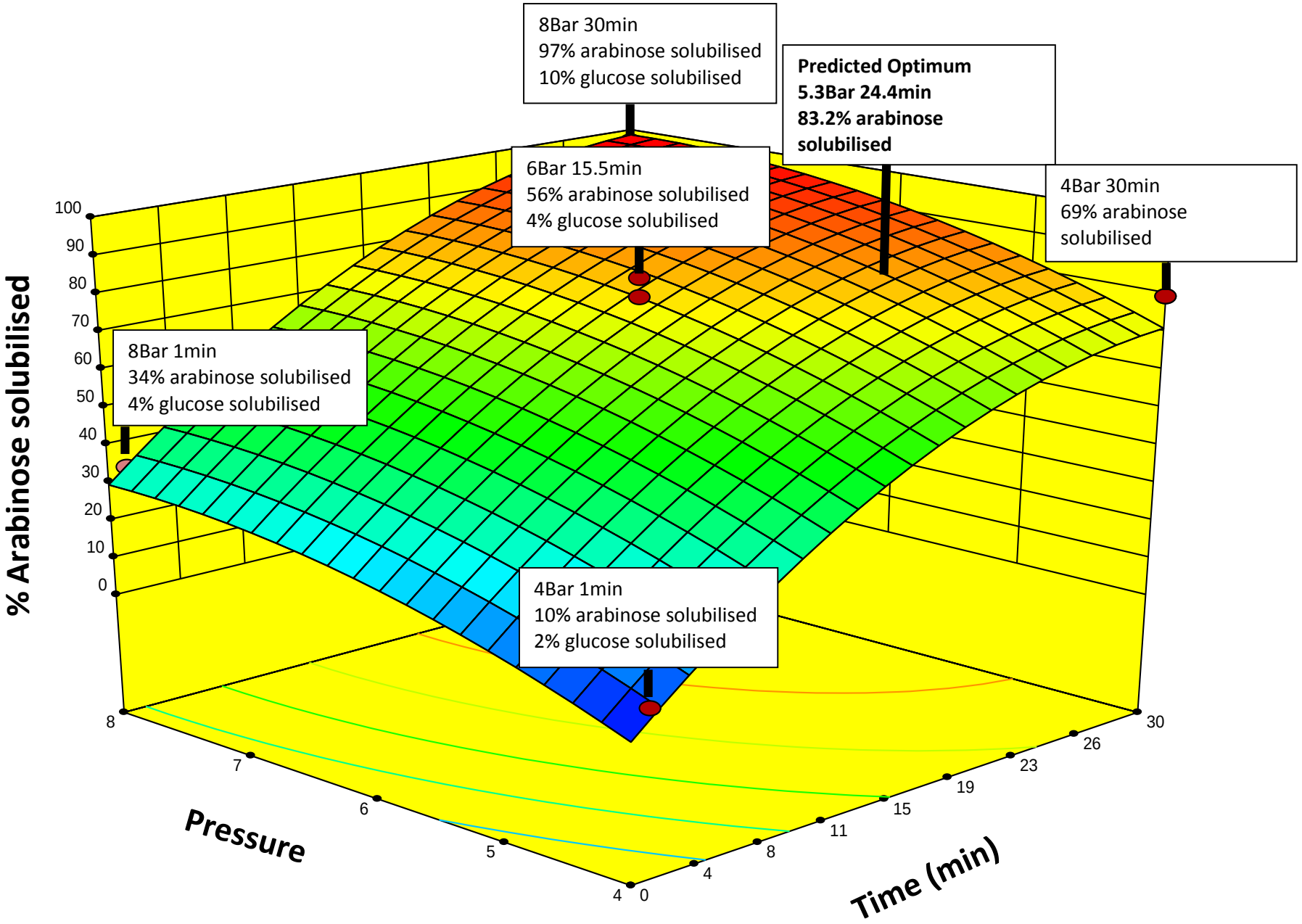
8Bar 30min
97% arabinose solubilised
10% glucose solubilised
Predicted Optimum
5.3Bar 24.4min
83.2% arabinose
solubilised
6Bar 15.5min
56% arabinose solubilised
4% glucose solubilised
4Bar 30min
69% arabinose
solubilised
8Bar 1min
34% arabinose solubilised
4% glucose solubilised
4Bar 1min
10% arabinose solubilised
2% glucose solubilised
% Arabinose solubilised
100
90
80
70
60
50
40
30
20
10
0
Pressure
8
7
6
5
4
Time (min)
0
4
8
11
15
19
23
26
30

Figure 2: Analysis of SBP fractions produced under predicted optimal conditions

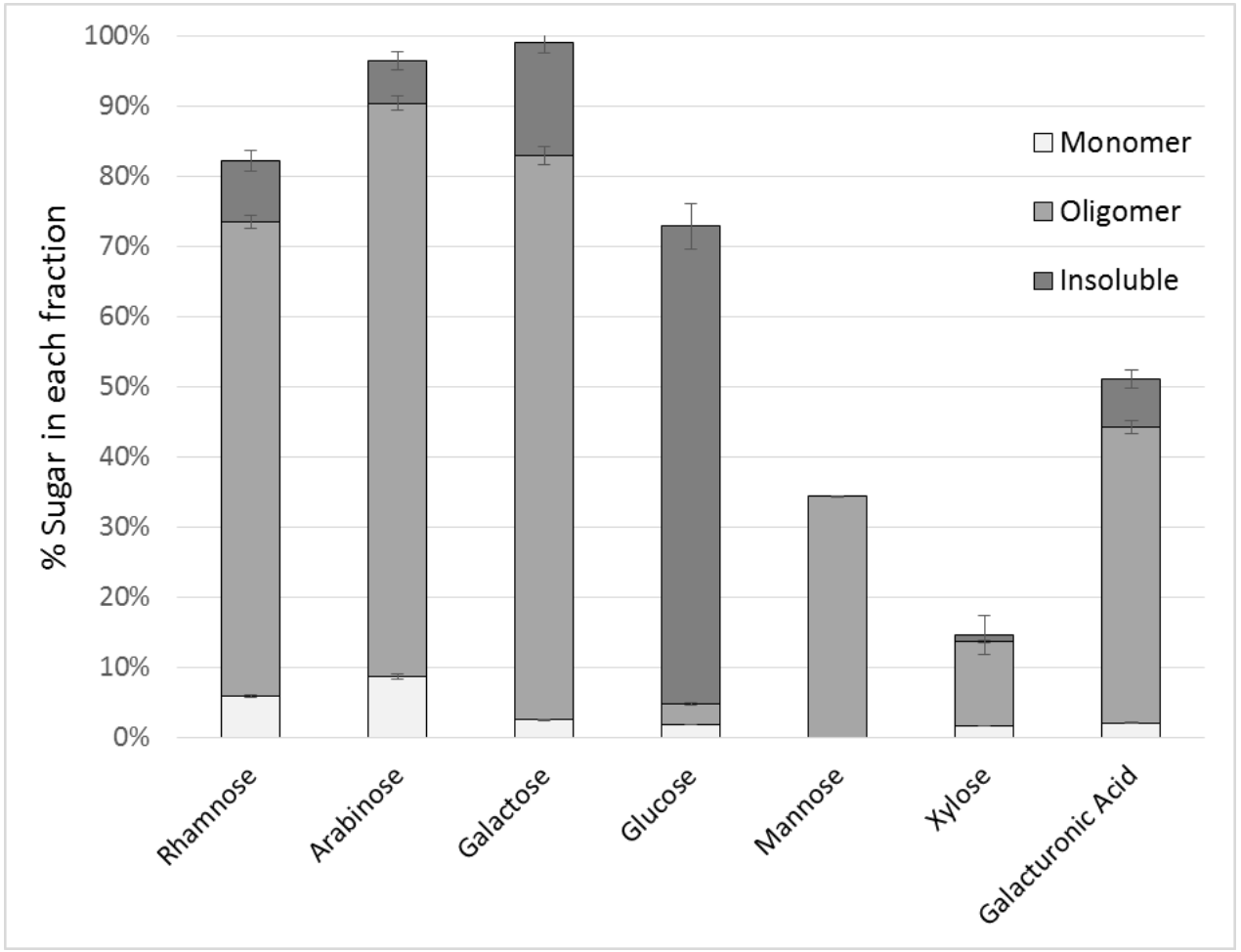

Table 6: Optical density of yeast cultures after 24hrs growth measured at 600nm. The residual carbohydrate and ethanol concentrations were determined by ion chromatography and HPLC as described in section 2.3.2. The conversion yield of glucose to ethanol is also shown.

	OD_{600}	Glucose consumed (mg)	Ethanol produced (mg)	Conversion g/g
SBP	5.28 (±0.10)	155.87 (±1.0)	75.26 (±0.12)	0.48 (±0.008)
YNBG	5.98 (±0.12)	134.39 (±4.0)	51.43 (±0.18)	0.38 (±0.013)

Table 7: Results of protein extraction performed on samples of raw untreated SBP, the insoluble residue after optimised steam pre-treatment and the residue after cellulase hydrolysis as described in section 2.4. Steam pre-treatment results in a loss of around 1% w/w protein, but the final insoluble product from cellulase hydrolysis is enriched in protein as the cellulose fraction is removed.

Sample	Dry weight of sample (mg)	ug protein in sample	%w/w
Washed SBP	106.9	8.69	8%
Steam pre-treated SBP	103.6	7.55	7%
Cellulase hydrolysed	119.0	18.02	15%